DATA EVALUATION RECORD
WHOLE SEDIMENT ACUTE TOXICITY INVERTEBRATES, FRESHWATER
OPPTS Guideline 850.1735

1. **CHEMICAL:** Permethrin PC Code No.: 109701

2. **TEST MATERIAL:** Permethrin technical Purity: 95.1%

3. **CITATION:**

Authors:	Picard, C.R.
Title:	10-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to Permethrin Applied to Formulated Sediment Under Static-Renewal Conditions.
Study Completion Date:	June 29, 2010
Laboratory:	Springborn Smithers Laboratories 790 Main Street Wareham, MA 02571-1037
Sponsor:	Pyrethroid Working Group Beveridge & Diamond 1350 I Street NW Washington, DC 20005
Laboratory Report ID:	13656.6138
MRID No.:	48593614
DP Barcode:	396414

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, CSS-Dynamac Corporation

Signature: Christie E. Padova **Date:** 11/25/13

APPROVED BY: Teri S. Myers, Senior Scientist, CDM Smith

Signature: Teri S Myers Date: 05/12/15

5. **APPROVED BY:**

Signature: **Date:**

6. **STUDY PARAMETERS:**

Age of Test Organism:	8 days old
Definitive Test Duration:	10 days
Study Method:	Flow-through

Type of Concentrations: Mean-measured sediment, bulk and OC-normalized

7. CONCLUSIONS:

Results Synopsis:

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 52 µg ai/kg 95% C.I.: 39 to 76 µg ai/kg
Probit Slope: 2.17 (1.44 to 2.89)
NOAEC: 26 µg ai/kg
LOAEC: 53 µg ai/kg

Growth (dry weight):
EC_{50}: 47 µg ai/kg 95% C.I.: 40 to 54 µg ai/kg
Probit Slope: N/A
NOAEC: 7.4 µg ai/kg
LOAEC: 13 µg ai/kg

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 2261 µg ai/kg TOC 95% C.I.: 1696 to 3304 µg ai/kg TOC
Probit Slope: 2.17 (1.44 to 2.89)
NOAEC: 1130 µg ai/kg TOC
LOAEC: 2304 µg ai/kg TOC

Growth (dry weight):
EC_{50}: 2043 µg ai/kg TOC 95% C.I.: 1739 to 2348 µg ai/kg TOC
Probit Slope: N/A
NOAEC: 322 µg ai/kg TOC
LOAEC: 565 µg ai/kg TOC

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable/Supplemental/Unacceptable

B. Rationale:

C. Repairability:

9. MAJOR GUIDELINE DEVIATIONS:

There were no deviations from OPPTS 850.1735 guidance that would affect the scientific soundness or acceptability of this study.

10. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *H. azteca* or *Chironomus tentans*	Amphipod crustacean, *Hyalella azteca*
Life Stage: For *C. tentans*: third instar (9-11 days old). The instar stage of midges must be confirmed by head capsule width (approx. 0.38 mm). For *H. azteca*: 7- to 14-day old amphipods must be produced. If growth is also an endpoint, a narrower range, such as 1- to 2-day old amphipods should be used.	8 days old
Supplier Brood stock can be obtained from laboratory, commercial, or government sources. (Sources obtained from the wild should be avoided unless cultured through several generations in the laboratory.)	Amphipods originated from laboratory cultures that were maintained in 20-L glass aquaria containing 15 L culture water under flow-through conditions. Both the culture water and overlying water used during the test originated from the same source.
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period: The required culture and testing temperature is 23°C. The test organisms should be cultured in the same water to be used for testing.	Adults were removed from the main culture tanks 9 days prior to test initiation and placed in *ca.* 8 L of water. Juvenile amphipods (≤24 hours old) produced by the isolated adults were then transferred to *ca.* 0.80 L of laboratory dilution water and reared for 8 days under static conditions at 23 to 24°C and 6.4 to 8.5 mg/L dissolved oxygen with gentle oil-free aeration.
Feeding:	During holding and acclimation, amphipods were fed every other day with 2.5 mL of a combination of yeast, cereal leaves, and flaked fish food suspension (YCT) and 2.5 mL of *Ankistrodesmus falcatus*.
Pretest Mortality: A group of organisms should not be used if they appear unhealthy, discolored (eg <20% mortality 48 h before the beginning of a test).	No mortality during the 48 hours prior to test initiation.

C. Test System

Guideline Criteria	Reported Information
Source of dilution water (overlying water) and sediment: Soft reconstituted water or water from a natural source. Tap water is acceptable if it is dechlorinated, deionized, and carbon filtered, but its use is not encouraged. Uncontaminated natural sediment is recommended.	Laboratory well water characterized as having a total hardness and total alkalinity as $CaCO_3$ of 64 to 66 mg/L and 19 to 21 mg/L, respectively, a pH range of 6.4 to 7.5, and a specific conductivity range of 410 to 450 µmhos/cm. The TOC of the dilution water source was 0.49 mg/L for January 2010. Formulated sediment (Springborn Smithers Batch No. 111909) was prepared according to OECD Guideline 218 by mixing the following components (dw basis): 6.0% sphagnum peat, 20% kaolin clay, and 74% fine sand. While blending using a large-scale mixer, 6 L of laboratory well water was also added. Prior to use, the sphagnum peat was pre-soaked in dilution water for 6 days. During this time, the peat was amended with 71 g of powdered $CaCO_3$ to increase the pH from 3.3 to 6.3.
Does water support test animals without observable signs of stress?	Yes
Quality Of Water If problems are observed in culturing or testing of organisms, it is desirable to test water quality. Particulate, TOC, COD should be <5 mg/L and residual chlorine <11 µg/L	There were no apparent problems with water quality. On Day 0, the measured ammonia level (as N) in control sediment pore water was 6.2 mg/L.

Guideline Criteria	Reported Information
Water Temperature 23°C for both species. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1°C and 3°C, respectively.	Daily and continuous: 22 to 24°C
pH Should not vary more than 50%. Survival is best at pH >6.5 for *C. tentans.*.	6.9 to 7.3
Dissolved Oxygen Maintained between 40 and 100%.	6.3 to 8.0 mg/L (>40% saturation)
Total Hardness Should not vary more than 50%. *H. azteca* are sensitive to hardness (e.g., they are not found in waters with calcium at <7 mg/L and DO at <2 mg/L).	64 to 72 mg/L as $CaCO_3$
Conductivity Should not vary more than 50%.	380 to 400 µmhos/cm
Sediment Characterization All sediment must be characterized for: pH, ammonia concentration of pore water, organic carbon content (total organic carbon (TOC)), particle size distribution, and percent water content.	Particle distribution – 80% sand, 3% silt, 17% clay (sandy loam; reviewer-derived from USDA soil texture triangle) TOC – 2.3% Percent solids – 68.82% pH – 7.1 CEC – not reported Bulk density – not reported
Additional Sediment Analysis BOD, COD, cation exchange capacity, Eh, pE, total inorganic carbon, total volatile solids, acid volatile sulfides, total ammonia, metals, synthetic organic compounds, oil and grease, petroleum hydrocarbons, and interstitial water analysis.	Not reported

Guideline Criteria	Reported Information
Laboratory Spiked Sediment Material should be reagent grade unless prior evaluations dictate formulated materials, etc.; Must know the test material's identity, quantity of major ingredients and impurities, water solubility, estimated toxicity, precision and bias of analytical method, handling and disposal procedures.	Permethrin technical Synonyms: FMC 33297, Pounce® IUPAC name: 3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate CAS name: (3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate Description: not reported Lot no.: PL07-0347 CAS No.: 52645-53-1 Purity: 95.1% Storage: room temperature in the dark Aqueous solubility: not reported
Stock Solutions Test material should be dissolved in a solvent prior to mixing into test sediment; If solvent is used, both solvent control and negative control are required.	A 25-µg ai/mL primary stock solution was prepared by dissolving 0.00263 g test substance (0.00250 g ai) into 100 mL of acetone. From this, six individual dosing solutions were prepared by diluting the appropriate amount of stock solution into 25 mL acetone. All dosing solutions were clear and colorless, with no visible un-dissolved test substance. Negative and solvent controls were included in the test.

Guideline Criteria	Reported Information
Test Concentrations For Spiked Sediment For LC50 calculation, test concentrations should bracket the predicted LC50; sediment concentrations may be normalized to factors other than dry weight (e.g. organic content, acid volatile sulfides); Sediment may be mixed using rolling mill, feed mixer or hand mixer.	A jar-rolling technique was used to apply the test substance to the sediment. A 10-mL volume of the appropriate prepared dosing stock solution (in acetone) was applied to 0.050 kg of fine silica and the solvent was allowed to evaporate off for 45 minutes. The dry sand was then added to 2.5 kg of wet sediment (total of 1.7705 kg dw) in individual glass jars. Each jar was then rolled for 4 hours at *ca*. 15 rpm. The jars were stored upright at 2 to 8°C for a 15-day equilibration period. Twice a week during the equilibration period and prior to being added into the replicate exposure vessels, the jars were mixed on the rolling mill for 2 hours to ensure the sediment was homogeneous. The range of nominal concentrations (4.0 to 128 µg ai/kg) was based upon the results of a preliminary range-finding study.
Test Aquaria 1. Material: Glass or stainless steel or perfluorocarbon plastics. 2. Size: 300 ml high-form lipless beakers containing 100 ml of sediment and 175 ml of overlying water.	1. Glass and 40-mesh Nitex screen (for drainage) 2. 300 mL vessels containing 100 mL (*ca*. 4.0-cm layer) of sediment (equivalent to 101 g dw) and 175 mL of overlying water. The total overlying water plus sediment volume was maintained at *ca.* 275 mL.
Type of Dilution System Daily renewal or a flow-through system may be used.	Flow-through
Flow Rate 2 volume changes/day	2 volume additions/day

Guideline Criteria	Reported Information
Aeration Dilution water should be vigorously aerated prior to use so that dissolved oxygen in the overlying water remains above 40% saturation.	None reported
Photoperiod 16 hours light, 8 hours dark at 500 to 1000 lux.	16 hours light, 8 hours dark; 500 to 710 lux
Solvents Use of a solvent should be avoided since they may influence the concentration in pore water. If used,it should not exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents include triethylene glycol, methanol, ethanol, or acetone. Surfactants should not be used.	Acetone, 10 mL per 1.7705 kg dw sediment The acetone was allowed to completely evaporate during the mixing procedure.

D. Test Design

Guideline Criteria	Reported Information
Sediment Into Test Chambers One day prior (Day -1) to start of test: test sediment, reference sediment, and negative control sediment should be thoroughly homogenized and added to test chambers; Overlying water is added to chambers in a manner that minimizes suspension of sediment.	One day prior to the addition of amphipods (day -1), the test systems were established. Overlying water was gently added, and each vessel was placed under the renewal system.

Guideline Criteria	Reported Information
Renewal of Overlying Water: Renewal of the overlying water should be conducted on day -1 prior to the addition of organisms or food on day 0. For flow-through systems, the flow rates should not vary by more than 10% between any two chambers at any time. Proper operation should be verified by calibration prior to test initiation.	The overlying water was renewed via an intermittent delivery system in combination with a calibrated water-distribution system. The test system was calibrated before and after the test, and visually inspected at least twice daily for proper functioning.
Placing Organisms in Test Chambers: Should be handled as little as possible and introduced into overlying water below the air-water interface.	Amphipods were impartially assigned one or two at a time into intermediate test beakers until all beakers contained ten amphipods. The test was initiated when each intermediate beaker of amphipods was added to each respective test vessel.
Range Finding Test A definitive test will not be required if no toxicity is observed at concentrations of 100 mg/kg dry weight of sediment**.**	<u>Preliminary toxicity assessment</u> • 10-day exposure at nominal levels of 0 (negative and solvent controls), 0.50, 5.0, 50, 500, and 5000 µg ai/kg • three replicates per level, each containing 10 organisms • Survival averaged 97 (negative control), 93 (solvent control), 100, 90, 47, 0, and 0%, respectively • Dry weight averaged 0.12 (negative control), 0.13 (solvent control), 0.13, 0.14, and 0.04 mg per amphipod, respectively
Monitoring the test All test chambers should be checked daily and observations made to assess organism behavior such as sediment avoidance.	Test vessels were observed daily for mortality and abnormal behavior.

Guideline Criteria	Reported Information
Nominal Concentrations of Definitive Test Control(s) and at least 5 test concentrations; dilution factor not greater than 50%. Concentrations above aqueous solubility may be used.	0 (negative and solvent controls), 4.0, 8.0, 16, 32, 64, and 128 µg ai/kg dw
Number of Test Organisms 10 organisms per test chamber are recommended. 8 replicates per treatment should be used.	80 amphipods per level, with 10 amphipods per replicate vessel and 8 biological replicates per level An additional 6 replicates per level were maintained for chemical analysis and pore water quality and analysis. The surrogate vessels did not contain organisms.
Test organisms randomly or impartially assigned to test vessels?	Yes
Feeding *C. tentans* in each test chamber are fed 1.5 ml of a 4 g/L Tetrafin7 suspension daily. *H. azteca* may be fed with a mixture of yeast, Cerophyl, and trout chow (YCT) at a rate of 1.5 mL daily per test chamber. A drop in DO levels below 2.5 mg/L may indicate over-feeding and feeding should be suspended in all treatments until DO levels increase.	Amphipods were fed a yeast, cereal leaves, and flaked fish food suspension (YCT) once daily at a rate of 1.0 mL/vessel.

Guideline Criteria	Reported Information
Water Parameter Measurements Conductivity, hardness, pH, alkalinity, and ammonia should be measured in all treatments at the beginning and end of the test. DO should be measured daily. Temperature should be measured daily in one test chamber from each treatment. The mean and instantaneous temperatures should not vary from the desired temperature by more than 1 and 3°C, respectively.	Total hardness, alkalinity, specific conductance, and ammonia were measured in each treatment level and control solution from a composite sample at Days 0 and 10. Dissolved oxygen (DO), temperature, and pH were measured in each replicate vessel on Days 0 and 10, and in one alternating replicate from each level on Days 1 to 9. In addition, the temperature was continuously monitored in an auxiliary vessel in the temperature-controlled water bath.
Chemical Analysis Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used. Concentrations should be measured in bulk sediment, interstitial water, overlying water, and stock solution.	Sediment from all levels was analyzed for permethrin on Days 0 and 10. Following removal of the overlying water, the sediment was centrifuged at *ca*. 1200 *g* for 15 to 30 minutes and extracted and analyzed using GC/MS based on methodology validated at Springborn Smithers (see Reviewer's Comments section for further details).

11. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes (see Reviewer's Comments).
Control Criteria Was control mortality ≤30%? Were control *C. tentans* an average size of ≥0.6 mg?	 Negative control – 3% Solvent control – 5% N/A
Percent Recovery of Chemical:	Results of quality control (QC) samples analyzed concurrently with test samples: Sediment: 104 to 116% of nominal at the 16.0 and 125 µg ai/kg levels, and 59.5 and 68.7% of nominal at the 2.00 µg ai/kg level.
Data Endpoints - Survival - Dry weight (determined by pooling all living organisms from a replicate and drying at 60 to 90°C to a constant weight) - Body length (amphipod only)	 - Survival - Dry weight
Raw data included?	Yes, sufficient

Effects Data

Toxicant Concentration				Survival		Dry weight per amphipod	
Nominal (µg ai/kg)	Mean-Measured[a]						
	Sediment (µg ai/kg)	Pore Water (µg ai/L)	Overlying Water (µg ai/L)	% ± SD	% Inhibition	mg ± SD	% Inhibition
Negative Control	<LOQ[b]	Not reported	Not assessed	97 ± 5	N/A	0.13 ± 0.01	N/A
Solvent Control	<LOQ	Not reported	Not assessed	95 ± 5	N/A	0.12 ± 0.01	N/A
Pooled Control	---	---	---	96 ± 5	N/A	0.13 ± 0.01	N/A
4.0	3.4	Not reported	Not assessed	94 ± 5	2.1	0.12 ± 0.02	7.7
8.0	7.4	Not reported	Not assessed	91 ± 7	5.2	0.12 ± 0.01	7.7
16	13	Not reported	Not assessed	99 ± 4	-3.1	0.11 ± 0.01*	15
32	26	Not reported	Not assessed	93 ± 12	3.1	0.09 ± 0.02*	31
64	53	Not reported	Not assessed	56 ± 13*	42	0.06 ± 0.02[c]	54
128	100	Not reported	Not assessed	8 ± 9*	92	0.02 ± 0.02[c]	85

[a] Results of the pore water analysis were not included in the study report and will be presented in a supplemental report, as stated by the study author. Overlying water was not analyzed in this study due to pyrethroids' strong affinity to sediment and regular renewal of the overlying water.
[b] LOQ = 0.41 to 0.54 µg ai/kg.
[c] Excluded from statistical analysis due to significant effect on survival at this level.
* Statistically-significant compared to the pooled control ($p<0.05$).

Other Significant Results:

Biological: After 10 days, survival averaged 97 and 95% for the negative and solvent controls, respectively, and 94, 91, 99, 93, 56, and 8% for the mean-measured 3.4, 7.4, 13, 26, 53, and 100 µg ai/kg sediment levels, respectively. Differences were statistically-significant compared to the pooled control (96%) at the 53 and 100 µg ai/kg treatment levels ($p<0.05$). Using mean-measured concentrations, the NOAEC and LOAEC for survival were 26 and 53 µg ai/kg, respectively, and the 10-day LC_{50} (with 95% C.I.) was 60 (53 to 66) µg ai/kg.

Mean dry weights per amphipod were 0.13 and 0.12 mg for the negative and solvent control levels, respectively, and 0.12, 0.12, 0.11, 0.09, 0.06, and 0.02 mg for the mean-measured 3.4, 7.4, 13, 26, 53, and 100 µg ai/kg sediment levels, respectively. Differences were statistically significant compared to the pooled control (0.13 mg/amphipod) at the 13 and 26 µg ai/kg treatment levels ($p<0.05$). Higher treatment levels were not statistically assessed due to significant reductions in survival at these levels. Using mean-measured concentrations, the NOAEC and LOAEC for growth were 7.4 and 13 µg ai/kg, respectively, and the 10-day EC_{50} (with 95% C.I.) was 46 (34 to 59) µg ai/kg.

Analytical: Only results from sediment analyses were reported in this study. Permethrin concentrations changed slightly (-23 to 27% , reviewer-calculated) during the 10-day study. Overall, mean-measured concentrations represented 79 to 92% of nominal sediment concentrations.

B. Statistical Results

Statistical analyses were performed on amphipod survival and growth (dry weight). Analyses were performed using the response values for each replicate test vessel within a treatment level. Percent survival data were arcsine square-root transformed prior to analysis.

A t-Test was used to compare the performance of the negative control and solvent control data. Survival and growth data were statistically similar, and the treatment groups were compared to the pooled control data to determine potential treatment-related effects.

Data were test for normality using the Chi-Square Test and for homogeneity of variance using Bartlett's Test (99% level of certainty). Survival and growth data met these assumptions and were thus analyzed using Bonferroni's Test (95% level of certainty). NOAEC and LOAEC values were assigned based upon significance.

The linear interpolation method was used to calculate the LC/EC_{50} values and associated 95% confidence intervals. All analyses were performed using TOXSTAT Version 3.5 statistical software and mean-measured sediment concentrations.

Survival:
LC_{50}: 60 µg ai/kg 95% C.I.: 53 to 66 µg ai/kg
NOAEC: 26 µg ai/kg
LOAEC: 53 µg ai/kg

Growth (dry weight):
EC_{50}: 46 µg ai/kg 95% C.I.: 34 to 59 µg ai/kg
NOAEC: 7.4 µg ai/kg
LOAEC: 13 µg ai/kg

12. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer compared the negative and solvent control data using a two-sided t-test; no significant difference was detected for survival or dry weight. Normality was tested using the Shapiro-Wilk test for normality and for homogeneity of variance using the Bartlett test for equality of variance. Survival and dry weight met both of these assumptions. The NOAEC and LOAEC for both suggestively dose-dependent endpoints were determined using William's Multiple Comparison Test. All treatment level comparisons were made to the negative control response only. The LC_{50} value was determined using the Probit method, while the EC_{50} for growth was determined using nonlinear regression. Results were provided in terms of mean-measured sediment (bulk and OC-normalized, calculated based on 2.3% TOC) in the Conclusions section of the DER. Analyses were conducted using CETIS v. 1.8.7.12 with backend settings implemented by EFED on 3/25/14.

Based upon mean-measured sediment concentrations:

Survival:
LC_{50}: 52 µg ai/kg 95% C.I.: 39 to 76 µg ai/kg
Probit Slope: 2.17 (1.44 to 2.89)
NOAEC: 26 µg ai/kg
LOAEC: 53 µg ai/kg

Growth (dry weight):
EC_{50}: 47 µg ai/kg 95% C.I.: 40 to 54 µg ai/kg
Probit Slope: N/A
NOAEC: 7.4 µg ai/kg
LOAEC: 13 µg ai/kg

Based upon OC-normalized mean-measured sediment concentrations:

Survival:
LC_{50}: 2261 µg ai/kg TOC 95% C.I.: 1696 to 3304 µg ai/kg TOC
Probit Slope: 2.17 (1.44 to 2.89)
NOAEC: 1130 µg ai/kg TOC
LOAEC: 2304 µg ai/kg TOC

Growth (dry weight):
EC_{50}: 2043 µg ai/kg TOC 95% C.I.: 1739 to 2348 µg ai/kg TOC
Probit Slope: N/A
NOAEC: 322 µg ai/kg TOC
LOAEC: 565 µg ai/kg TOC

13. REVIEWER'S COMMENTS:

The reviewer's conclusions generally agreed with the study author's. It should be noted that the study author's results were reported with respect to the pooled control, while the reviewer reported results as compared to the negative control only.

Dosing stock solutions and treated sediment from all levels (prior to allocation into the replicate vessels) were analyzed for permethrin. Recoveries in the stock solutions ranged from 84 to 110% of nominal concentrations. Analysis of the spiked sediment following dosing and prior to allocation into the replicate exposure vessels ranged from 72 to 96% of nominal concentrations.

The analytical method used to quantify permethrin in formulated sediment was validated on April 30 to May 5, 2009. Fortified samples were extracted two to three times with methanol:purified reagent water and hexane; the extracts were combined and purified for analysis using solid phase extraction (SPE). Aliquots were analyzed using gas chromatography equipped with mass selective detection in negative chemical ionization mode (GC-MS/NCI). The method validation established an average recovery of 93.1 ± 8.02% (CV=8.61%) for permethrin from formulated sediment fortified at 1.00 and 1000 µg ai/kg. The limit of quantitation (LOQ) was 0.475 µg ai/kg. A method validation extension was conducted in February 2010, which established an average recovery of 102 ± 4.88% (CV=4.78%) for permethrin from formulated sediment fortified at 20,000 µg ai/kg.

Overlying water was not analyzed due to the pyrethroids' strong affinity to sediment (i.e., high K_{oc} values) and regular renewal of the overlying water. Documentation supporting that only negligible amounts of pyrethroids partition to overlying water were cited (Springborn Smithers Laboratories Study Nos. 13656.6106, 13656.6107, 13656.6110, 13656.6111, and 13656.6112; Putt, 2005).

It was reported that data for permethrin concentrations in pore water and bulk sediment for pore water will be presented in a supplemental report.

In addition to total hardness and specific conductivity, total alkalinity and ammonia were determined in the overlying water of each level on Days 0 and 10. Total alkalinity ranged from 20 to 22 mg/L as $CaCO_3$, and ammonia (as N) ranged from ≤0.10 to 0.49 mg/L.

The redox potential, pH, ammonia, dissolved organic carbon (DOC), and total organic carbon (TOC) were measured in a pore water sample obtained from each test level on Days 0 and 10. The redox potential ranged from 190 to 230 mV, the pH ranged from 6.7 to 7.0, TOC and DOC were 120 to 180 mg C/L and 94 to 150 mg C/L, respectively, and the ammonia content (as N) decreased from 6.1 to 7.6 mg/L on Day 0 to 1.4 to 1.9 mg/L on Day 10.

This study was conducted in compliance with all pertinent U.S. EPA GLP regulations (40 CFR, Part 160) with the following exceptions: routine water, sediment, and food contaminant screening analyses. These analyses were performed using certified laboratories and standard validated methods.

Definitive test dates were January 8 to 18, 2010.

14. REFERENCES:

APHA, AWWA, WEF. 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition, American Public Health Association, American Water Works, Association and Water Environment Federation, Washington, DC.

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Drive, West Conshohocken, PA 19428.

Ditsworth, G.R., D.W. Schults, and J.K.P. Jones. 1990. Preparation of Benthic Substrates for Sediment Toxicity Testing. *Environmental Toxicology and Chemistry.* Vol. 9: 1523-1529.

European Commission. 2000. Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. Working document. Directorate General Health and Consumer Protection. SANCO/3029/99 rev. 4, 11/07/00.

OECD, 2004. OECD Guideline for Testing of Chemicals. Sediment Water Chironomid Toxicity Test Using Spiked Sediment. Guideline #218. Adopted 13 April 2004.

Putt, A.E., 2005. Bifenthrin – Toxicity to Midge (*Chironomus tentans*) During a 10-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6106.

Putt, A.E., 2005. Bifenthrin – Toxicity to Estuarine Amphipods (*Leptocheirus plumulosus*) During a 28-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6107.

Putt, A.E., 2005. Cypermethrin – Toxicity to Midge (*Chironomus tentans*) During a 10-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6110.

Putt, A.E., 2005. Cypermethrin – Toxicity to Estuarine Amphipods (*Leptocheirus plumulosus*) During a 28-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6111.

Putt, A.E., 2005. Cypermethrin – Life-Cycle Toxicity Test with Midge (*Chironomus tentans*) During a 60-Day Sediment Exposure. Springborn Smithers Laboratories, Wareham, MA. Study No. 13656.6112.

Sokal, R.R., and F.J. Rohlf. 1981. *Biometry.* 2nd Edition. W.H. Freeman and Company, NY. 859 pp.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Test Guideline, OPPTS 850.1735. Whole Sediment Acute Toxicity Invertebrates, Freshwater, "Public Draft" EPA 712-C-96-354 April 1996. U.S. Environmental Protection Agency. Washington, D.C.

U.S. EPA. 2000. Office of Water. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates. Test Method 100.4. EPA/600/R-99/-64. March 2000. U.S. Environmental Protection Agency. Washington, D.C.

U.S. EPA. 2008. Ecological Effects Test Guidelines, OPPTS 850.1735 Spiked Whole Sediment 10-Day Toxicity Test, Freshwater Invertebrates. U.S. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. EPA 712-08-354.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide, and Rodenticide Act. Good Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, DC.

Weber, C.I., *et al.* (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd edition. EPA/600/4-89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency,

Cincinnati, OH.

West, Inc. and David D. Gulley. 1996. TOXSTAT Version 3.5. Western Ecosystems Technology, Inc. Cheyenne, Wyoming.

Zumwalt, D.C., *et al.* 1994. A water-renewal system that accurately delivers small volumes of water to exposure chambers. *Environmental Toxicology and Chemistry.* 13: 1311-1314.